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厦门大学

硕　士　学　位　论　文

高静水压对葡萄牙牡蛎胚胎和幼体发育的影响

The effects of high hydrostatic pressure on the development of embryos and larvae of *Crassostrea angulata*

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缩略词表

英文缩写	英文名称	中文名称
APS	ammonium persulfate	过硫酸胺
bp	base pair	碱基对
BSA	bovine serum albumin	牛血清白蛋白
cDNA	complementary DNA	互补 DNA
cDNA-AFLP	cDNA amplified restriction fragment length polymorphism	cDNA 扩增限制性片段长度多态性
ddH_2O	double distilled H_2O	双蒸水
DEPC	diethypyrocabonate	焦乙酸二乙酸
dNTP	deoxy-ribonucleoside triphosphate	三磷酸脱氧核糖核苷
dsDNA	double-stranded DNA	双链 DNA
DTT	diethyl pyrocarbonate	二硫苏糖醇
EDTA	ethylene diamine tetraacetic acid	乙二胺四乙酸
EST	expressed sequence tag	表达序列标签
fs cDNA	First-stranded cDNA	第一条链 cDNA
GO	gene ontology	基因本体数据库
HHP	high hydrostatic pressure	高静水压
HSP	heat shock proteins	热休克蛋白
IPTG	Isopropyl β-D-1-Thiogalactopyranoside	异丙基-β-D-硫代吡喃半乳糖苷
KEGG	Kyoto Encyclopedia of Genes and Genomes	京都基因与基因组百科全书
MID	Multiplex Identifier	多样标识符
MIPS	the Munich Information Center for Protein Sequences	慕尼黑蛋白质序列信息中心
miRNA	microRNA	小 RNA
mRNA	messenger RNA	信使 RNA

英文缩写	英文名称	中文名称
MSAP	Methylation sensitive amplified polymorphism	甲基敏感扩增片段多态性
OD	optical density	光密度
PA	polyacrylamide	聚丙烯酰胺
PBS	phosphate-buffered saline	磷酸盐缓冲液
rDNA	ribosomal DNA	核糖体 DNA
RNA-seq	RAN sequencing	RNA 测序
RT-PCR	reverse transcription polymerase chain reaction	逆转录聚合酶链式反应
SAGE	serial analysis of gene expression	基因表达系列分析
SAM	S-acid armour sulfur acid atp	S - 腺苷酰甲硫氨酸
SDS	sodium dodecyl sulfate	十二烷基磺酸钠
SEM	scanning electron microscope	扫描电镜
siRNA	small interfering RNA	小的干扰 RNA
TAE	Tris-acetic- EDTA(buffer)	Tris/醋酸/EDTA 缓冲液
TBE	Tris-borate-EDTA(buffer)	Tris/硼酸/EDTA 缓冲液
TEMED	N,N,N'N'-tetramethylethylenediamine	N,N,N'N'-四甲基乙二胺
Tris	Tris(hydroxylmethyl) amino-methane	三羟甲基氨基甲烷

摘要

高静水压(High Hydrostatic Pressure，HHP)是一种极端的物理压力效应, 存在于压强超过 10MPa 的液体中，它相当于 1000m 水深所产生的压强，可以对细胞产生多种影响，包括染色体的结构、膜的整合性、DNA 和蛋白的相互作用及改变基因的表达等。除深海生物外，高压也会影响常压生物的正常生活。在高压环境下，生物体会产生相应的对应机制，以适应极端环境，有关高静水压对生物生长影响的研究目前仅限于从海洋中分离的细菌、真菌以及部分陆生动植物。在水产动物方面，高静水压更多的被运用于鱼类及贝类的遗传育种，如三倍体育种等。

在本文，我们研究了高静水压对葡萄牙牡蛎胚胎及幼体发育的影响，这些信息能够帮助我们更好的理解高压环境下生物体适应和生存机制。主要研究结果如下：

1、本文应用正交试验的方法设计了 3 因素 3 水平的 9 组试验，同时设立在常压下生长的对照组，利用高静水压仪在牡蛎发育的几个关键时期实施压力诱导，研究静水压对于牡蛎胚胎和幼体发育的影响。在水温 25℃，盐度 30 的条件下，3 个实验因子及 3 个实验水平分别是：牡蛎早期不同的发育阶段(囊胚期、担轮幼虫期、D 型幼虫)；高压的持续时间(10、30、50min)；静水压强的大小(10、20、40MPa)。以牡蛎受精后第 12 天的成活率和第 19 天的平均体长为指标，分析了 9 个实验组牡蛎幼体的成活率及体长。结果显示，以牡蛎受精后第 12 天的成活率为指标，三个因子重要性顺序是：压力大小>高压持续时间>开始处理时间；最佳处理条件是：在牡蛎幼体发育至 D 型幼虫时，施以 10MPa 的压力 50min，有望获得较高成活率的幼体。若以受精后第 19 天的平均体长为指标，三个因子重要性顺序是：开始处理时间>高压持续时间>压力大小；最佳处理条件是：在牡蛎幼体发育至担轮幼虫时，施以 10MPa 的压力 30min，有望获得生长较快的幼体。对高静水压处理前后的葡萄牙牡蛎胚胎和幼体进行染色体制片及倍性分析，结果显示，各个压力实验组与对照组在染色体数目及结构均无显著差异，说明在本实验的静水压处理条件下，静水压对染色体畸变的影响较小。对牡蛎幼体原生和次生贝壳进行扫描电镜观察，结果显示，受精后 36h，第 2 组(囊胚

期、30min、20MPa)，第 3 组(囊胚期、50min、40MPa)和第 4 组(担轮幼虫期、10min、40MPa)的个体看不到明显的 D 型壳，大小明显小于其他 7 组的幼体个体大小，可见高静水压能够改变牡蛎幼体从受精卵到 D 形幼虫的发育快慢；实验组与对照组受精后 3 天和眼点期幼虫的形态比较结果，未发现有显著的差异。综上，不同的高静水压处理条件不仅能够提高牡蛎幼体成活率和生长速度，也能导致幼体发育滞后甚至死亡。在实际生产中，该实验结果可以用于指导水产养殖生产实践，有利于提高幼体的成活率和生长速率。

2、利用甲基化敏感扩增多态性技术(methylation sensitive amplified polymorphism, MSAP)，对葡萄牙牡蛎幼体高静水压处理前后的基因组 DNA 5’-CCGG-3’位点进行全基因组甲基化扫描检测。聚丙烯酰胺凝胶电泳及银染结果表明，与对照组相比，虽然在静水压处理前后相同遗传背景材料中获得了多个甲基化位点的差异，但是经过高静水压处理后的 DNA 甲基化条带比率仅由对照组的 9.4%增加至 10%，差异不显著。我们尚不能判断甲基化是否参与静水压对牡蛎幼体的互作，结果尚需进一步的验证。

3、利用转录组数据进行基因表达差异分析。构建高静水压处理组第 9 组和对照组的 cDNA 文库，经过 454 测序、拼接和验证，拼接后共获得 13653 条序列，并对这些序列进行 GO 注释。选取在测序中被表达的次数在 20 以上的片段，共获得序列 275 条，其中被 GO 注释的有 170 条，为总序列的 61.8%，2 倍以上的上调序列有 26 条(占 15.3%)，2 倍以上的下调序列有 31 条(占 18.2%)。根据 GO 功能注释，2 倍以上的上调或下调基因主要涉及一些能量和代谢基因、转录和翻译调节基因、细胞的运输调节和信号转导基因、细胞防御和内环境的调节基因、细胞结构成分基因以及一些功能未知的基因。这些差异基因的上调或抑制表达可能对牡蛎幼体在高静水压下的适应和生存具有重要的作用。本研究首次对海洋贝类在高静水压诱导处理下的基因表达进行分析，基因组表达类型的功能分析揭示了高压特异的压力反应类型，提供了关于逆境环境下生物体适应和生存机制的丰富信息。

关键词：高静水压；葡萄牙牡蛎；胚胎和幼体；DNA 甲基化分析；转录组分析

Abstract

High hydrostatic pressure (HHP) is an extreme thermal physical pressure effect, and exists in more than 10MPa pressure of liquid. It is equivalent to 1000m depth of sea water. High hydrostatic pressure have different effects on cells, including the structure of the chromosomes, the integration of membrane, the interaction of DNA and proteins, and the changes in gene expression, etc. In addition, HHP can also affect the organisms in atmosphere pressure. At the same time, the organisms can form corresponding defense mechanism to adapt to high pressue. Many adaptation mechanism to HHP has been well studied in bacterias and fungus isolated from the deep sea, and some terrestrial organisms. In aquatic animals, HHP is mainly used in the genetic breeding of fish and shellfish, such as the triploid breeding.

The different effects of HHP on the development of embryos and larvae of *C. angulata* were studied. These informations could help to understand the pressure-adapataion mechanism for *C. angulata.*

1、Three factors and three levels of nine groups orthogonal test were designed. We designed the HHP instrument to carry out the pressure-induced experiment in several key stages of oyster development, and explored the effect of HHP on the development of embryos and larvae of *C. angulata*. Under the condition of water temperature 25℃ and the salinity 30, three factors and three levels were: different early development stages of *C. angulata* (blastocysts stage, trochosphere stage and D shape larvae); The duration of HHP (10, 30 and 50 min); The magnitude of HHP (10, 20 and 40 MPa). The osyter survival rates of the 12th day and the shell sizes of the 19th day after fertilization as indexes, the survival rates and shell sizes of larvae were analysed. The results showed that, 12th day after fertilization as the index, the magnitude was the most important; The optimal condition of treatment: the higher survival rate of the oysters was required in the stage of D shape larvae, under the pressure of 10MPa and duration of 50min. Nineteenth day after fertilization as the index, the stage of treatment was the most important; The optimal condition of treatment: the faster grow rate of the oysters was required in the stage of trochosphere, under the pressure of 10MPa and duration of 30min. Comparision of the chromosome of embryos and larvae of *C. angulata* between HHP and control groups, there were no significant differences. This indicated that HHP had little influence on the distortion of chromosome. By using scanning electron microscope for the primary and secondary shell of larval *C. angulata*, the results showed that the D shape shells of the larvae of group 2 (blastocysts stage, 30min and 20MPa), group 3 (blastocysts stage, 50min and 40MPa) and group 4 (trochophore, 10 min and 40 MPa) were not obvious, and the sizes of larvae were much smaller than other senven groups. It was indicated that HHP could affect the growth rate of larval *C. angulata* development from fertilized eggs to D shape larvae. There was no distinction in the comparison of morphology of larvae of the third day after fertilization and eye spot between HHP and control groups. In conclusion, different conditions of HHP could not only improve the survival and growth rate of *C. angulata*, but also induce development lag and even death. In practice, the results can be used to guide aquaculture and improve the survival and growth rate of *C. angulata*.

2、We scanned and deteced the whole genome DNA 5'-CCGG-3' methylation of larval *C. angulata* of HHP and control groups by using the method of methylation sensitive amplified polymorphism(MSAP). Compared with the control group, the ratio of DNA methylation of larvae after HHP treatment was only increased from 9.4% to 10% and the differences were not significant, although the differences of multiple methylation sites in the same genetic background materials of before and after HHP treatment. Whether the methylation participated in the interaction of HHP with larval *C. angulata* was unclear, and need further research.

3、We used transcriptome data to analyse the differences of gene expression, and constructed the cDNA librarys of HHP and control groups. We analysed GO annotation by the obtained 13653 sequences. Selecting the sequences which were showed more than 20 times in sequencing and 275 were acquired. Among these sequences, 170 were annotated and represented 61.8% of total. Twenty six genes (15.3% of total) were >2-fold induced while 31 (18.2% of total) suffered a > 2-fold

downregulation. According to GO annotation, the majority of the > 2-fold upregulated and repressed genes were involved in some energy and metabolic genes, transcription and translation regulated genes, cell transportation, signal path genes, cell defense and regulation of cellular environment, cell organization and some unknown genes. These differential genes may play an important role in the adaption and survival of *C. angulata* under HHP. The gene expression of the marine shellfish afer HHP induction treatment was firstly analysed. The functional analysis of genomic expression revealed a hydrostatic pressure-specific stress response pattern and provided abundant informations of the mechanisms involed in the adaptation and survival of organisms under extreme environment. These changes caused by HHP indicated that HHP treatment could change the expression of genes and induce the adaptive response of *C. angulata*.

Key Words: high hydrostatic pressue; *Crassostred angulata*; embryos and larvae; DNA methylation analysis; transcriptome analysis